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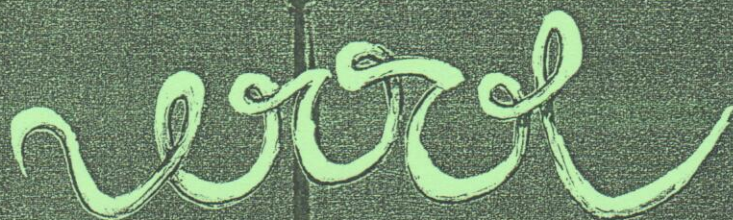
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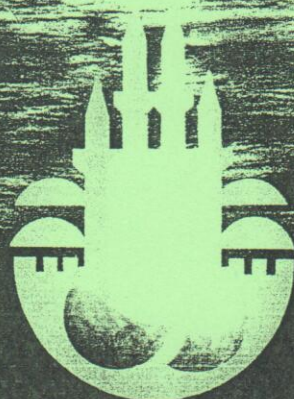


INTERNATIONAL WOOL TEXTILE ORGANIZATION

62nd INTERNATIONAL



CONFERENCE



16-21 May 1993

ISTANBUL-TURKEY





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Barcelona 2th April 1993

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Dear J. GRIGNET,

I am pleased to send you herewith enclosed the rapport concerning our paper entitled "Phospholipid Bilayers Including Cholesterol as Vehicles of Disperse àyes in Wool Dyeing" for presentation in the Technical Committee, to be held in Istambul the next 16th-21st of mai.

Thanking you for your cooperation,

Sincelely Yours,

Dr. A de la Maza  
Chemical Technology Dept

INTERNATIONAL WOOL TEXTILE ORGANISATION  
Technical Committee

ISTANBUL  
May 1993.  
Report nr 31

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## **PHOSPHOLIPID BILAYERS INCLUDING CHOLESTEROL AS VEHICLES OF DISPERSE DYES IN WOOL DYEING**

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### **ABSTRACT**

Studies on the use of multilamellar lipid vesicles (MLV) of defined size (400nm) containing cholesterol (CH) as carriers of disperse dyes to wool fibres are described. We investigated liposomes made up egg phosphatidylcholine (PC) containing the azo disperse dye C.I. Disperse Orange 1 at different PC/CH/relative concentrations. We assessed physical stability by measuring the mean vesicle size distribution of the vesicle suspensions after preparation and during the dyeing process. Kinetic aspects involving dye adsorption and bonding on untreated wool samples by means of MLV liposomes at different PC/CH ratios were also investigated. This process led to the controlled exhaustion of dye in wool samples, which was directly dependent on the relationship existing between PC and CH components, with a clear improvement both in the dye-fibre bonding forces and in the dispersing efficiency of these systems with respect to the use of conventional dispersing agents for this dye. The optimum application of these systems in the percentage of dye exhaustion and in the total amounts of bonded dye on untreated wool samples was reached for PC:CH molar ratios 8:2 for the dye/lipid weight ratio corresponding to the maximum level of dye encapsulation efficiency of these systems. This weight ratio was also correlated with the lipid composition of bilayers, decreasing as the cholesterol concentration in bilayers increased.

## INTRODUCTION

Many amphiphilic compounds have been used in the dyeing process as dispersing agents of water-insoluble disperse dyes. But few studies have been performed on the mechanisms of these interactions (1,2) and on the application of these systems on keratinic structures. The use of water-insoluble disperse dyes increased considerably with the advent of synthetic fibres some of which (polyesters) are much more hydrophobic than the first man-made fibre on which these dyes were applied i.e., cellulose acetate and therefore very resistant to conventional water-soluble dyes (3).

Although disperse dyes have good affinity for protein fibres, they have not been used to date on wool dyeing process (4). The irregular staining and, in some cases, the poor wash fastness are the main disadvantages of these dyes for wool dyeing. The selection of the disperse dye, the dyeing conditions and the use of a suitable carrier is considered to be very important in preventing this effect which is particularly pronounced when dyeing wool/polyester blends (5).

Liposomes are aqueous dispersions organized as bilayers of lipid molecules widely used as a simplified model of biological membranes and as delivery systems where encapsulation and protection of hydrophilic and lipophilic substances are required (6-11).

Although initially slow to exploit the technology of liposomes, the textile industry has now produced a wide variety of innovations using the basic principles of targeting, slow release and protection of sensitive chemicals principally in the dyeing and the finishing of textiles (12-13). Including CH in bilayer membranes has a condensing effect and tends to retard protein penetration (14). The presence of this component is also a very important factor for obtaining liposome formulations that are both stable in biological environments and suitable as chemical carriers (15). Likewise, liposomes containing CH have been investigated as vehicles of the oxidative reagent in wool chlorination (16).

We previously reported PC liposomes as carriers in commercial dyeing of untreated wool using milling acid dyes and disperse dyes (12,17).

In the present paper, bearing in mind that cholesterol is one of the main components of the internal lipids of wool (18), we have studied the effects caused by including this component in lipid bilayers to obtain improved applications in wool dyeing using disperse dyes. To this end, we describe work on the physical stability of multilamellar liposomes (MLV) containing

the azoic disperse dye C.I. Disperse Orange 1 at different PC/CH molar ratios the dye concentration remaining constant. The application of these structures in dyeing of untreated wool fibres has also been examined and their application on untreated wool samples, focusing on the kinetic aspects of dye adsorption and the dye-fibre bonding forces on wool fibres.

## **EXPERIMENTAL**

### **Materials**

Botany wool fabrics knitted from R64/2 tex (count 2128) yarns were used. Samples were Soxhlet extracted for 2 hours with methylene chloride and rinsed with water purified by the Milli-Ro system (Millipore) and dried at room temperature.

The azo-disperse dye (C.I. Disperse Orange 1) was supplied by Sigma Chemical Co. (St. Louis) and shown to be pure by thin-layer chromatography (TLC). The dye chemical structure is given in Figure 1. This dye was selected as a representative azoic disperse dye which is hardly soluble in water (0.1 mg dye/l at 25°C) needing a high concentration of surface-active assistant to be dispersed (1% of sodium oleyl-p-anisidide sulphonate solution (Lissapol LS) is needed to disperse 41 mg of dye per liter of water) (3).

Phosphatidylcholine (PC) was purified from egg lecithin (Merck) according to the method of Singleton (19) and shown to be pure by thin-layer chromatography (TLC). Cholesterol (CH) was purchased from Sigma Chemical Co. (St. Louis, MO). Lipids were stored in chloroform under nitrogen at -20°C until use.

Polycarbonate membranes of 400 nm and 800 nm, and membrane holders used for liposome extrusion were purchased from Nucleopore (Pleasanton, CA).

The nonionic surfactant Triton X-100 (octylphenol with 10 units of ethylene oxide and active matter of 100%) was specially prepared by Tenneco S.A. (Barcelona, Spain).

### **Preparation of Multilamellar Vesicle Liposomes (MLV)**

Multilamellar vesicle liposomes of a defined size (400 nm) at different lipid concentrations (from 1.25 mM to 3.0 mM), varying the lipid composition (PC/CH molar ratios from 10:0 to 8.0:2.0) respectively, the dye concentration remaining constant (1.0 mM) were prepared

following a method described by Banghman (20).

A film was formed by removing the organic solvent from chloroform/methanol 2:1 solution of egg PC, CH and dye solutions by rotary evaporator in a nitrogen atmosphere and low vacuum (350 mm Hg). An aqueous phase containing sodium sulphate 5%, and acetic acid (pH 5.5) was then added to the film formed. The solutions were then swirled to remove the lipid from the walls of the flask and to disperse large lipid/dye aggregates; glass beads were added to facilitate dispersions. The resulting milky suspensions were vortexed for 5 minutes and sonicated for 15 minutes at 30°C and 75W (Labsonic 1510 B. Braun). Liposome suspensions were extruded through 800 and 400 nm polycarbonate membranes to obtain a uniform size distribution (21). After preparation the resulting liposome suspensions were left to equilibrate for 15 minutes and immediately applied in wool dyeing processes.

### Dyeing Procedure

Wool knitted samples were treated with MLV liposome suspensions freshly prepared at different PC/CH molar ratios (from 10:0 to 8:2) in the range of lipid concentrations from 1.25 mM to 3.0 mM, the dye concentration remaining constant (1 mM which corresponded to 1.9 % o.w.f.). The dye was applied with 5% o.w.f. anhydrous sodium sulphate, acetic acid at pH 5.5 and liquor ratio 60:1.

Dyeing was started at 50°C and the temperature was raised by 0.9 °C/ min to 90°C. Dyeing was continued for 120 min. Thereafter, samples were rinsed with water for 10 min and dried at room temperature. Laboratory dyeing was carried out in a Multi-Mat dyeing machine (Renigal).

Dyebath exhaustion was determined by spectrophotometry using a Shimadzu UV-265FW spectrophotometer. Liposome aliquots (0.5 ml) were periodically added to quartz cuvettes filled with 2 ml of aqueous solution of Triton X-100 (2% w/v), supplemented with sodium sulphate (5%). The interaction between the nonionic surfactant Triton X-100 and the liposomal structures resulted in a solubilization of lipid vesicles via mixed micelles formation (22,23), turning the liposome suspensions into a clear solution.

Figure 2 shows the effect of the cleavage of liposome vesicles by Triton X-100 on the absorption spectra of the Disperse Orange 1 dye at different PC/CH molar ratios (from 10:0 to 8:0). It may be seen that the  $\lambda_{max}$  of the dye used in this study does not change in presence

of increasing amounts of CH in the lipid/surfactant mixed micelles

### Determination of the Encapsulation Efficiency and Mean Vesicle Size Distribution of Liposome Vesicles

The maximum amounts of dispersed dye via MLV liposomes given as the weight ratio between the dispersed dye and the lipids in bilayers will be defined in this paper as K. This ratio was determined by spectrophotometry. After preparation, liposome suspensions were left to equilibrate for 12 hours. Afterwards, vesicle suspensions were spun at 5000 r.p.m. at 25° for 15 min in order to remove the unencapsulated dye. Finally, the concentration of dispersed dye was evaluated by spectrophotometry after the destruction of the supernatant lipid bilayers by addition of Triton X-100 (22,23).

Mean vesicle size and polydispersity of the liposome preparations were determined by a Photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). The studies of the particle size distribution were made by particle number measurements. Samples were adjusted to the appropriate concentration range. The measurements were made at 25°C with a detection angle of 90°.

### Aggregation Measurements

The aggregation state of the vesicles was estimated as a measure of the physical stability of the liposome suspensions. This was done by monitoring the variation of the mean vesicle size distribution of liposome suspensions as a function of time.

### Bilayer Lipid Composition

Lipid composition of different liposome vesicles studied was determined using the Iatroscan MK-5 TLC-FID analyser. Coupling thin-layer chromatography (TLC) to an automated detection system based on flame ionization detection (FID) is a recent innovation (24), which has considerably improved the sensitivity of TLC and allows quantitation of separated materials. This method has been used to quantify most kinds of lipids from different sources (25).



Previous experience on lipid analysis from queratinized tissues such as wool (26) lead us to choose this procedure to quantify these particular lipid mixtures even then are forming liposomes in water solutions.

### Dye extraction

The superficial dye bonded on the fibres by non polar forces (hydrophobic interactions, van der Waals forces and hydrogen bonds) was extracted with pure ethanol at 25°C for 60 min (12). Subsequent extractions with ammonia solution (0.5% at 60°C for 15 min) stripped the dye diffused inside the fibre and bonded ionically (3).

## RESULTS AND DISCUSSION

### Encapsulation Efficiency of MLV Liposomes

The variation of the total amounts of the Disperse Orange 1 dye dispersed via MLV liposomes at different bilayer lipid compositions (PC:CH from 10:0 to 8:2 molar ratios), versus liposome lipid concentration is indicated in Figure 3. It may be seen that a linear dependence can be established between both parameters in all cases. The weight ratio dye/lipid corresponded to the slope of the straight lines obtained.

Table I shows the K values obtained for the different PC:CH molar ratios used as well as the regression coefficients of the straight lines obtained. It is noteworthy that K values increase as the CH concentration in bilayers decreases reaching the highest value for PC:CH molar ratio 10:0 ( K 0.29). It is interesting to note that the use of MLV liposomes resulted in an clear improvement in the dye dispersion efficiency (from 70 times to 63 times, depending on the CH concentration in bilayers), with respect to the use of dispersing agent normally used for this dye (3)

### Stability of Liposome suspensions

The possible aggregation of liposomes during the dyeing process was monitored by

measuring the variations in the mean vesicle size distribution of these suspensions using a quasi-elastic light scattering method (27). The results obtained for liposome suspensions at different lipid compositions (PC:CH molar ratios from 10:0 to 8:2 and total lipid concentration 2 mM) and using in each case the corresponding maximum weight ratio value (Table I) are given in Table II.

It may be observed that there is a small increase in the particle size distribution during dye, the polydispersity indexes remaining below 0.15 after treatment. It is noteworthy that increasing amounts of CH in liposomes enhance the stability of these systems with respect to the aggregation, reducing both the mean particle size distribution values and the polydispersity indexes during the dyeing. This behaviour is in agreement with the results reported by Scherphof et al. in studies on liposome stability (28). Likewise, the mean vesicle size distribution was maintained at around 400 nm and the polydispersity index below 0.24 for more than 24 hours.

### **Dyeing Kinetics**

We carried out kinetical studies of dye exhaustion for these systems on untreated wool samples at lipid PC:CH compositions ranging from molar ratios 10:0 to 8:2 also varying the total lipid concentration of bilayers (from 1.25 mM to 3.0 mM), the dye concentration remaining constant in all cases (1 mM). The results obtained are plotted in Figure 4.

Figure 4-A plots the exhaustion kinetics of the Disperse Orange 1 dye via MLV liposomes at lipid composition PC:CH molar ratio 10:0. It may be seen that dye exhaustion increases as the lipid bilayer concentration increases. The highest point was reached for 1.50 mM lipid concentration (final dye exhaustion 88,7%). Increasing lipid concentration in bilayers resulted in a decreased dye exhaustion, the minimum being obtained for 3 mM lipid concentration.

Figures 4-B and 4-C also plots the exhaustion kinetics of the same dye for MLV liposomes at PC:CH 9:1 and 8:2 inolar ratios, respectively . The experiments were made varying the lipid composition in the same range than showed in Fig 4-A, the dye concentration remaining also constant (1mM). It may be seen that the dye exhaustion also increased as the lipid concentration increased, the highest level being obtained in both cases ai 1.75 mM lipid concentratioii. Increasing lipid concentration in liposomes also resulted, in all cases, in a fall in the dye exhaustion

Comparing the results showed in Figures 4(A-C) it is interesting to note that increasing amounts of cholesterol in lipid bilayers resulted in a slightly decrease of the maximum dye exhaustion in wool samples. Thus, the PC:CH 10:0 molar ratio led to 88.7% of dye exhaustion whereas PC:CH 8:2 molar ratio resulted only in a exhaustion value of 79.2%. Likewise, the dye/lipid weight ratio (K) at which these systems reached the maximum dye exhaustion corresponded approximately to the maximum encapsulation efficiency of these systems

From optical microscope observations of dyes samples, a regular dye distribution was observed in all case and especially using liposome suspensions at the PC/CH molar ratios corresponding to the maximum dye exhaustion. Increasing amounts of CH in bilayers also improved the dye distribution on untreated wool samples.

### **Influence of Liposome composition on the bonding of Dyes on wool**

In order to find out whether liposomes (containing increasing amounts of CH) as dye carriers caused changes to dye-fibre bonding forces after dyeing, extractions by pure ethanol (12) and ammonia (3) were performed on dyed samples. The results of dye extractions are given in Tables III, IV and V. In general terms, pure ethanol extracted, in all cases, larger amounts of dye than ammonia solutions. This could be attributed to the high solubility of this dye in pure ethanol as well as to the presence of dye superficially bonded on the fibres by non-polar forces. However, the very small amounts of dye extracted via ammonia respect to the lipid and dye concentrations of liposomes could be attributed to the important contribution of the non-polar forces in the dye-fibre bonds. These interactions, especially those that are hydrophobic in nature, may play an important role in the dye-fibre bonding inside the fibre. Bearing in mind the data, it should be pointed out that increasing amounts of lipids in liposomes, the dye concentration remaining constant (1mM) resulted in a decreased dye extraction via pure ethanol, being these extracted amounts inversely dependent on the CH concentration in bilayers, i.e., the lower the CH concentration in bilayers the higher the dye extraction via pure ethanol. However, ammonia extractions showed very low values in all cases.

Tables III, IV and V also show the amount of bonded dye in untreated wool fibres given as a difference between the amounts of the adsorbed dye and total extracted dye. As regards

the data, it is noteworthy that the maximum amounts of bonded dye via liposomes were obtained, in each case, at the lipid/dye molar ratio corresponding to the maximum dye exhaustion. (1.50 mM for PC:CH 10:0 molar ratio and 1.75 mM for both PC:CH molar ratios 9:1 and 8:2), the highest amounts being obtained for the maximum CH concentration in bilayers (Table V).

Plotting the amounts of bonded dye versus lipid concentration for three levels of CH concentration graphs of Figure 5 are obtained. It may be seen that the straight lines obtained showed an inflexion point (points a,b and c) with a maximum which corresponded to the K values where these systems reached a maximum value of encapsulation efficiency.

The total percentage of bonded dye on wool fibres can be also expressed by the equation 1:

$$C_b = \frac{C_a - C_e}{C_e} \times 100 \quad (1)$$

where  $C_b$  is the relative amount of bonded dye (%),  $C_a$  is the amount of absorbed dye (mg dye per g wool) and  $C_e$  is the total amount of extracted dye (mg dye per g wool). These percentages are also given in tables III, IV and V.

In general, the higher the CH concentration in bilayers, the higher the percentages of bonded dye, the maximum percentages being also obtained at the K values corresponding to the maximum encapsulation efficiency of these systems.

In our opinion, the main contribution of this paper is the development of a new method to apply azo disperse dyes to wool samples via liposomes MLV including cholesterol in bilayers at controlled temperature (90°C). This process can be suitable for improving the dye bonding in wool fibres in the absence of the dispersing agents normally used for these dyes of wool (18). This technological innovation simplifies the method currently used with disperse dyes drastically reducing the lipid concentration needed to disperse the dye with respect to the dispersing agent concentration normally needed to obtain the same dispersing effect. Increasing amounts of CH in bilayers, leads to an improvement in the dye-fibre bonding forces, despite that results in a decrease in the total dye exhaustion on wool.

## CONCLUSIONS

From our findings, a new method of wool dyeing using the azo disperse dye C 1 Disperse



Orange 1 via MLV liposomes in presence of increasing amounts of CH could be considered suitable for the modulation of dye exhaustion improving the dye-fibre bonding forces and the dye distribution on wool samples

Liposomes progressively decreased their encapsulation efficiency<sup>3</sup> as the CH concentration in bilayers increased, the maximum weight ratio dye/lipid (K 0.29) being obtained for PC:CH molar ratio 10:0 and the minimum (K 0.26) for PC/CH molar ratio 8:2. From these findings, the use of MLV liposome suspensions resulted in an clear improvement in the dye dispersion efficiency ( from 63 to 70 times depending on the CH concentration in bilayers), with respect to the use of the conventional dispersing agents for this dye (3).

Liposome formed by PC:CH at differerit molar ratios were stable during the dyeing process at pH 5,5, the lipid concentrations ranging from 1.25 mM to 3.0 mM, and for up to 24 following preparation.

The dye exhaustion on untreated wool fibres was directly dependent on the lipid concentration of bilayers and on the bilayer lipid composition. The maximum values were obtained for the PC:CH molar ratio 10:0 (88.7%) at lipid concentration 1.5 mM (6.8% o.w.f.) and for the PC:CH molar ratios 9:1 (82.7%) and 8:2 (79.2%) at lipid concentration 1.75 mM (7.5% o.w.f. and 7.2 % o.w.f.) respectively, the dye concentration remaining constant (1.0 mM which corresponds to 1.9% o.w.f.).

The maximum amounts of bonded dye were obtained, for each liposome composition, at the K ratio corresponding to the maximum encapsulation efficiency of each system. Increasing amounts of CH in bilayers resulted in an increment in the total percentages of bonded dye in wool fibres.

### ACKNOWLEDGEMENTS

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## REFERENCES

1. Ogino, K., T. Kasuya and M. Abe,. Colloid Polym. Sci., **266**:539, (1988)
2. Abe, M., T. Kasuya and K. Ogino,. Colloid Polym. Sci., **266**:156, (1988)
3. Trotman, E.R. in "Dyeing and Chemical Technology of textile Fibres", Edited by Charles Griffin and Company Ltd., England, 1984. Chapter 12.
4. Brady, P.R., Rev. Prog. Coloration, **22**:58, (1992)
5. Wang, J. and H. Asnes, J.S.D.C., **107**:314, (1992)
6. Paternostre, M.T., M. Roux and J.L. Rigaud, Biochemistry, **27**:2668, (1988).  
Almog, B., J. Litman, W. Wimley, J. Cohen, E.J. Wachtel, Y. Barenholz, A. Ben-Shaul and D. Lichtenberg, Biochemistry, **29**:4582, (1990).
8. Chapman D. in "Liposome Technology", Vol. I, Edited by G. Gregoriadis, CRC. Press Inc. Boca Raton Flonda 1986 pp. 1-19.
9. de la Maza, A., J.L. Parra and J. Sanchez Leal, Langmuir, **8**:2422, (1992)
10. Levy, D., A. Gulik, M. Seigneuret, and J.L. Rigaud, Biochemistry, **29**:9480, (1990).
11. Inoue, T., T. Yamahata and R. Shimozawa, J. Colloid Interface Sci., **149**:345, (1992).
12. de la Maza, A., J.L. Parra, A. Manich and L., Coderch, J.S.D.C., **108**:540, (1992).
13. Nelson, G., Rev. Prog. Coloration, **21**:72, (1991)
14. Juliano.R.L., Interactions of Proteins and Drugs with Liposomes, in, "Liposomes" Edited by J.M. Ostro, Marcel Dekker, Inc. New York, 1983 Chapter 2.
15. Papahadjopoulos, D., M. Cowden and H.K. Kimelberg, Biochim. Biophys Acta, **330**:8, (1973).
16. de la Maza, A. and J.L. Parra, Textile Res. J. (in press)
17. de la Maza, A. and J.L. Parra, J. Amer. Oil Chem. Soc. (in press).
18. Rivett, D,E. , Structurai Lipids of the Wool Fibre, Wool Sci. Rev, **67**:1, (1991).
19. Singleton, W.S., M.S. Gray, M.L. Brown, and J.L. White, J. Amer. Oil Chem Soc., **42**, (1965), 53.

20. Bangham, A.D., M.M. Standish and J.C. Watking, *J. Mol. Biol.* 13:238, (1965)
21. Szoka, F., F. Olson, T. Heath, W. Vail, E. Mayhew and d. Papahadjopoulos, *Biochim. Biophys. Acta* 601:559, (1980).
22. Helenius, A., and K. Simons, *Biochim. Biophys. Acta*, 415:29, (1975)
23. Lichtenberg, D., J. Robson and E.A. Dennis, *Biochim. Biophys. Acta* 737:285, (1983).
24. Ackman, R.G., Flame ionization detection applied to thin-layer chromatography on coated quartz rods. in "Methods in Enzymology" edited by Lowenstein, J.L., Academic Press, New York, 1981, Vol 72, pp. 2205-252.
25. Ackman, R.G., C.A. McLead and A.K. Banejee, *J. of Planar Chromatography*, 3:450, (1990)
26. Coderch L., C. Sonano, A. Pinazo, J.L. Parra and P. Erra, *Textile Res. J.* 62:704, (1992).
27. Chong, C.S. and K. Colbow, *Biochim. Biophys. Acta* 426:260, (1976).
28. Scherphof, G.L., J. Damen and J. Wilschut, Interaction of Liposomes with Plasma Proteins, in "Liposome Technology" Vol III, edited by G. Gregoriadis, CRC Press, Inc. Boca Raton, Florida 1986, Chapter 14.

## FIGURE AND TABLE LEGENDS

FIGURE 1. Chemical structure of C.I. Disperse Orange 1 dye

FIGURE 2. Adsorption spectra of Disperse Orange 1 dye in presence of lipid/Triton X-100 mixed micelles at different bilayer lipid compositions (PC:CH molar ratios 10:0 (●), 9.5:0.5 (▲), 9:1 (▼), and 8:2 (■).

FIGURE 3. Maximum amounts of dispersed dye versus lipid concentration for three levels of CH in bilayers.

FIGURE 4. Plots the exhaustion kinetics of Disperse Orange 1 dye on untreated wool samples in dyeing via liposomes at different lipid concentrations (mM) 1.25 (a), 1.50 (□), 1.75 (●), 2.0 (○), 2.25 (▼), 2.50 (▽), 2.75 (▲), 3.0 (△), and different lipid compositions: (A) PC:CH 10:0, (B) PC:CH 9:1 and (C) PC:CH 8:2, the dye concentration remaining constant (1 mM).

FIGURE 5. Amounts of bonded dye in wool fibres versus lipid concentration in liposomes at different levels of CH in PC bilayers.

TABLE I. Weight ratios corresponding to the maximum encapsulation efficiency for liposomes containing increasing concentrations of CH in bilayers. The regression coefficients of the straight lines are also given.

TABLE II. Mean vesicle size distribution and polydispersity of MLV liposomes at different lipid compositions (PC:CH molar ratios from 10:0 to 8:2 and total lipid concentration 2 mM) during the dyeing process.

TABLES III, IV and V.

Amounts of adsorbed dye (mg dye/g wool), extracted dye (mg dye/g wool) and bonded dye (mg dye/g wool and %) in wool samples after dyeing via LMV liposomes at different lipid concentrations and different lipid compositions (TABLE III PC:CH 10:0 molar ratio, TABLE IV PC:CH 9:1 molar ratio and TABLE V PC:CH 8:2 molar ratio)



TABLE I

PC:Chol molar ratio	Weight Ratio K	Regression Coefficients $r^2$
10:0	0.29	0.994
9.0:1.0	0.27	0.993
8.0:2.0	0.26	0.992

TABLE II

Time min	Mean vesicle size			Polydispersity Index		
	PC:CH 10:0	PC:CH 9:1	PC:CH 8:2	PC:CH 10:0	PC:CH 9:1	PC:CH 8:2
0	404	401	398	0.092	0.084	0.085
5	402	400	398	0.092	0.082	0.084
10	400	399	395	0.094	0.084	0.086
15	398	396	394	0.120	0.110	0.099
30	402	395	392	0.123	0.114	0.102
45	410	400	398	0.130	0.118	0.106
60	418	416	405	0.132	0.121	0.110
75	422	418	409	0.134	0.122	0.114
90	425	419	411	0.142	0.129	0.122
105	431	423	415	0.144	0.134	0.128
120	435	429	417	0.150	0.141	0.130

TABLE III

Lipid Conc	Adsorbed dye	Extracted dye		Bonded Dye	
(mM)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
1.25	15.469	3.970	0.014	11.485	74.24
1.50	16.939	3.840	0.014	13.085	77.37
1.75	16.443	3.780	0.013	12.650	76.93
2.00	15.851	3.732	0.010	12.109	76.39
2.25	15.259	3.694	0.011	11.554	75.71
2.50	14.609	3.650	0.009	10.950	74.95
2.75	13.941	3.610	0.009	10.322	74.04
3.0	13.387	3.570	0.008	9.809	73.27

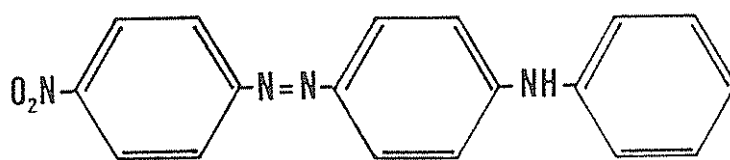
TABLE IV

Lipid Conc	Adsorbed dye	Extracted dye		Bonded Dye	
(mM)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
1.25	13.196	2.871	0.014	10.311	78.13
1.50	15.507	2.723	0.014	12.767	82.33
1.75	15.794	2.660	0.012	13.123	83.08
2.00	14.953	2.610	0.011	12.332	82.47
2.25	14.266	2.600	0.010	11.656	81.70
2.50	13.559	2.601	0.009	10.949	80.75
2.75	12.795	2.547	0.008	10.244	80.06
3.0	12.069	2.493	0.008	9.568	79.27

TABLE V

Lipid Conc	Adsorbed dye	Extracted dye		Bonded Dye	
(mM)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
	11.630	2.018	0.013	9.599	82.53
	14.418	1.904	0.014	12.500	86.69
	15.125	1.830	0.012	13.203	87.29
2.00	14.094	1.753	0.010	12.331	87.49
2.25	12.986	1.694	0.009	11.283	86.88
2.50	12.069	1.608	0.008	10.453	86.61
2.75	10.924	1.501	0.006	9.417	86.20
3.0	9.950	1.423	0.003	8.527	85.69

FIGURE 1



C.I. Disperse Orange 1



FIGURE 2

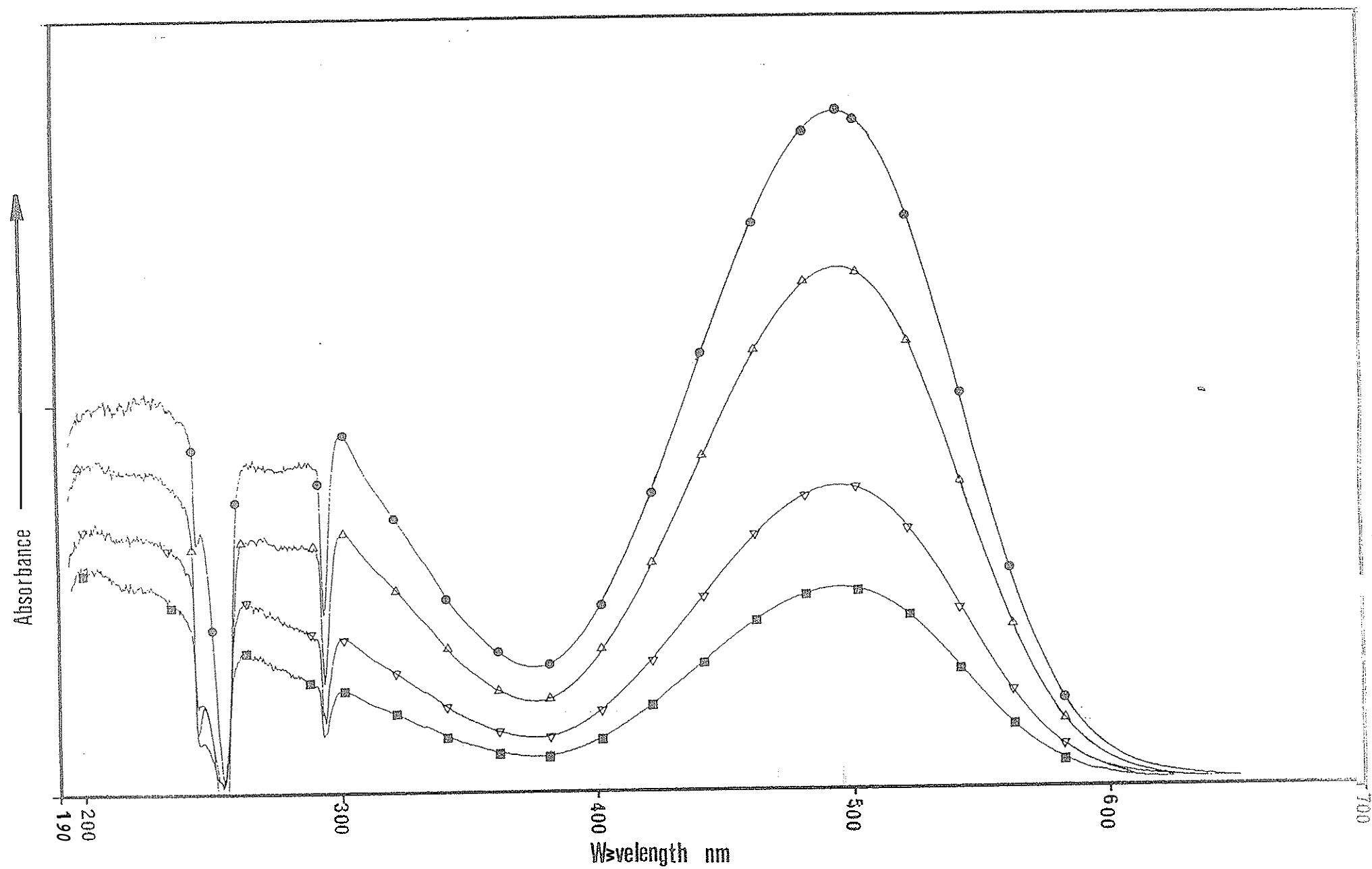
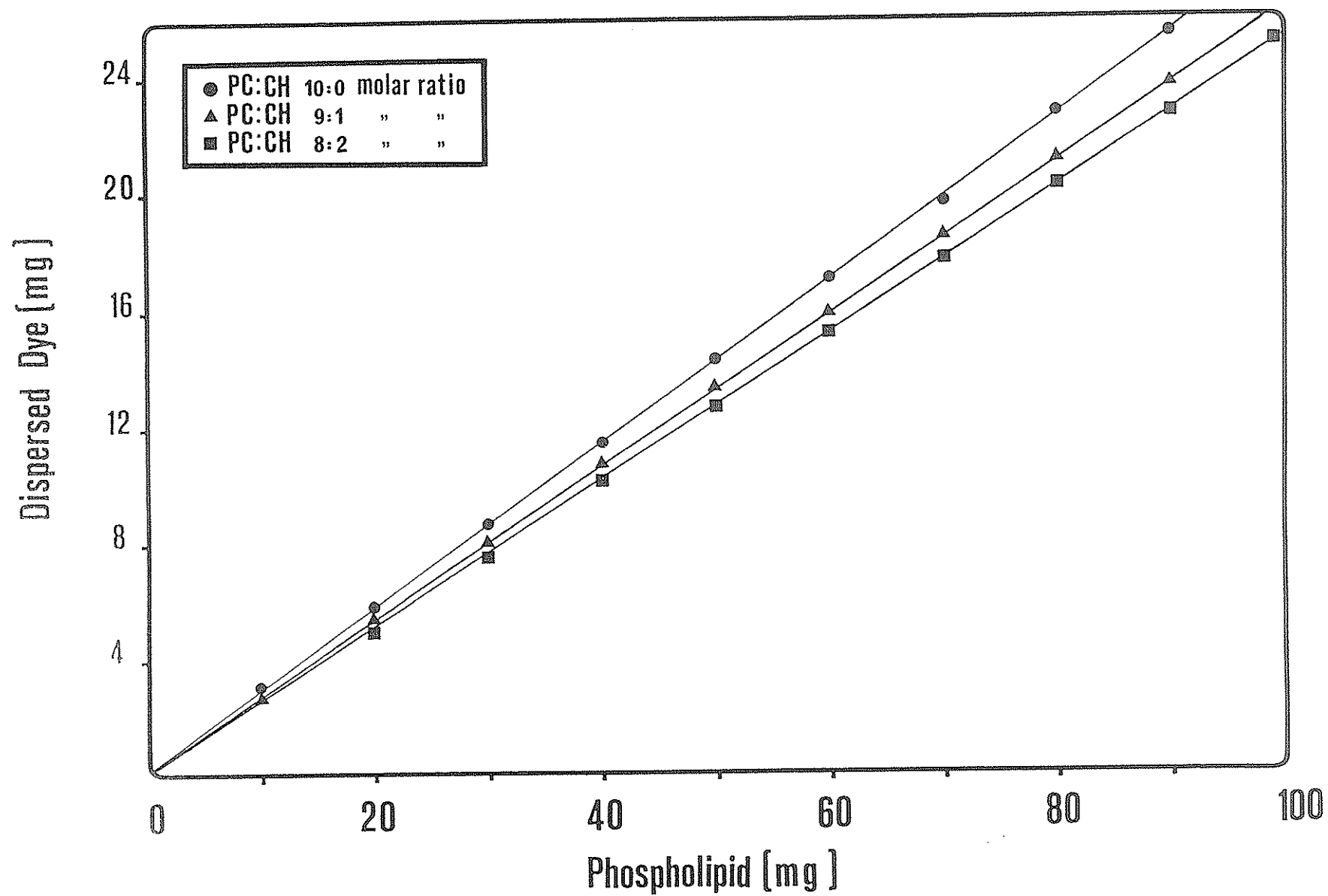


FIGURE 3



Exhaustion Dye

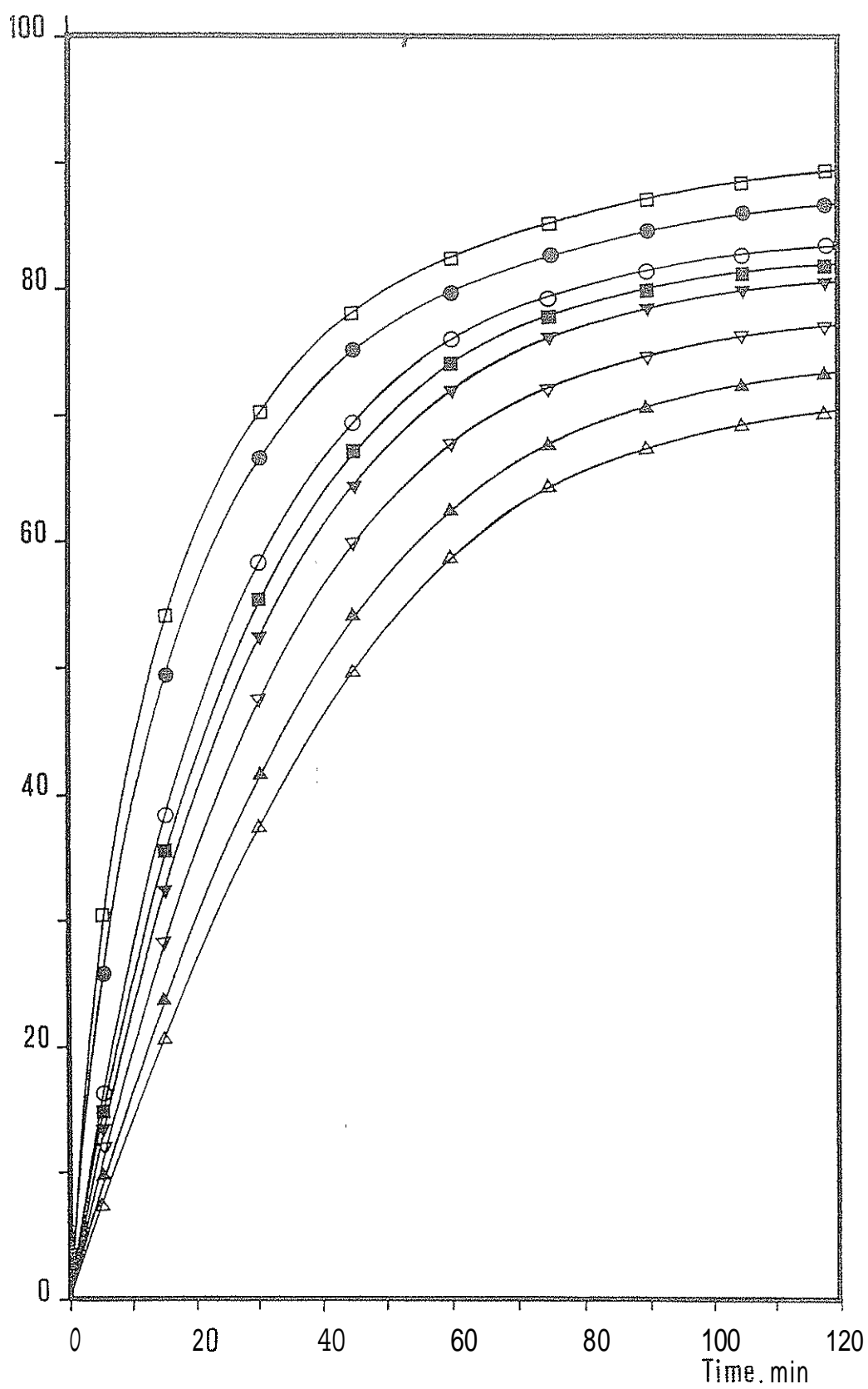


FIGURE 4-A

Exhaustion Dye

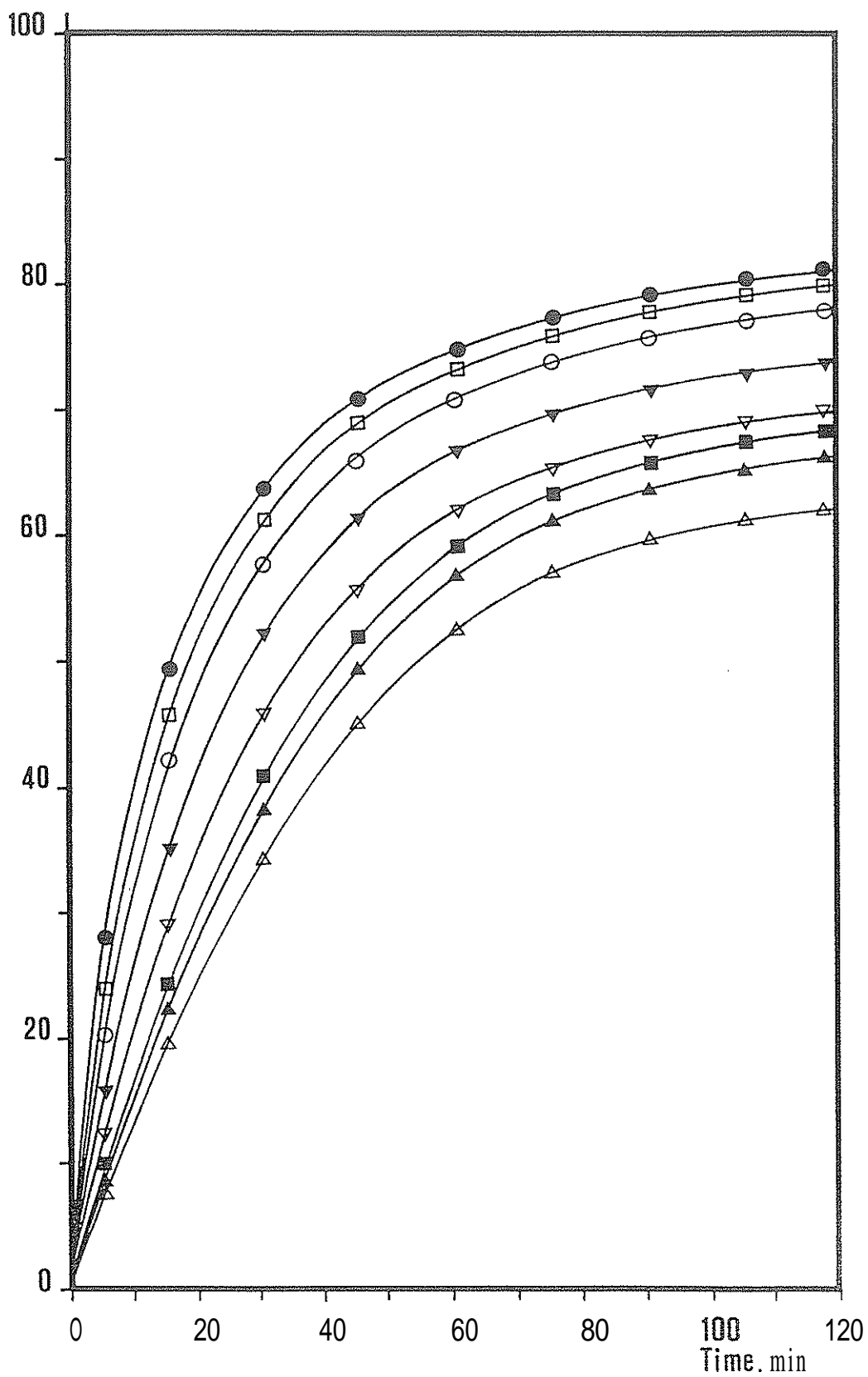


FIGURE 4-B

Exhaustion Dye

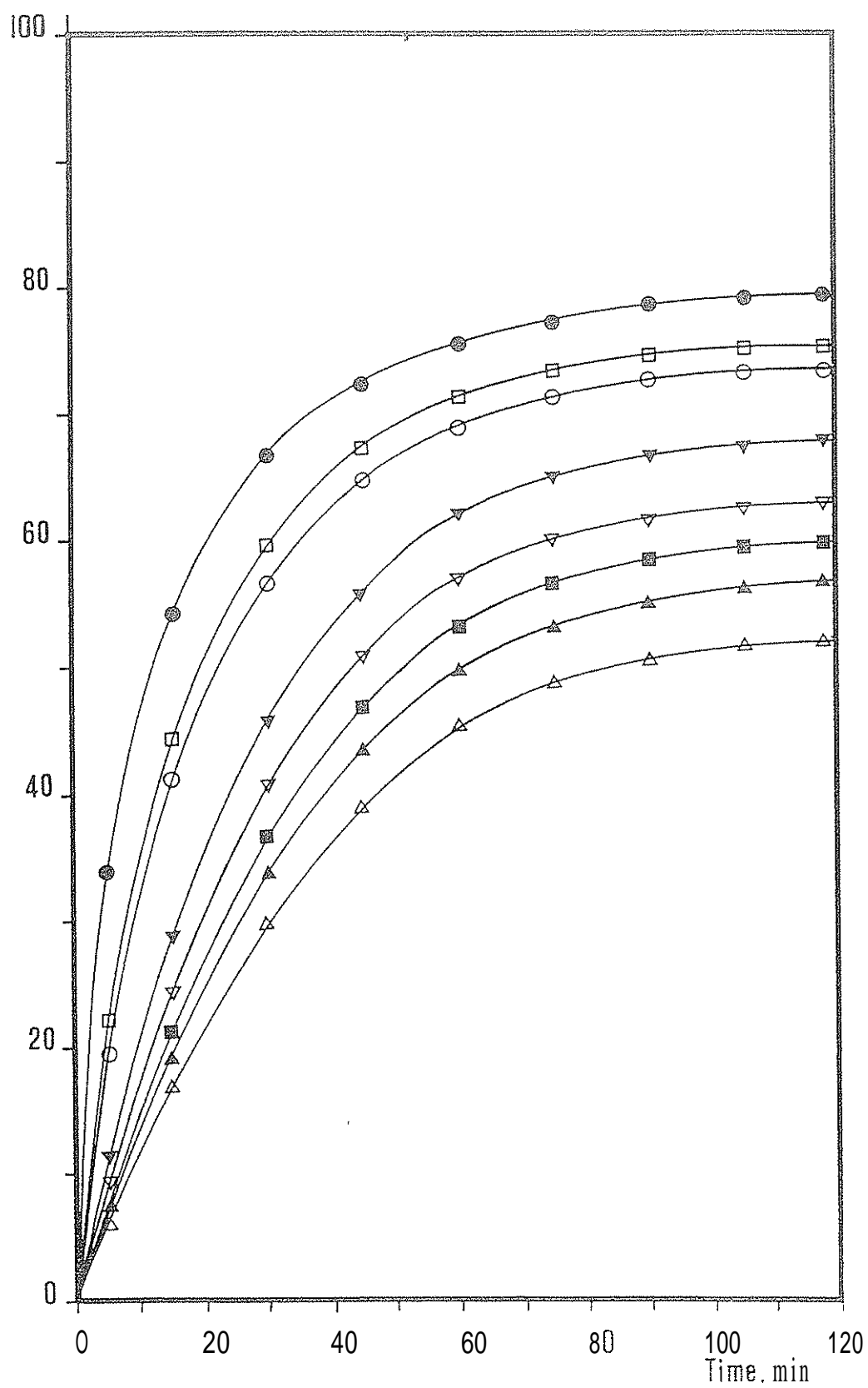


FIGURE 4--C



FIGURE 5

